

**23.** The method of claim **22**, wherein said oxidized amino acid residue is selected from group consisting of methionine, tryptophan, histidine, phenylalanine, tyrosine and a combination thereof.

**24.** The method of claim **22**, wherein said oxidized amino acid residue is selected from an amino acid residue on a polypeptide having an amino acid sequence as set forth in the group consisting of: SEQ ID NO.: 17, SEQ ID NO.: 18, SEQ ID NO.: 19, SEQ ID NO.: 20, SEQ ID NO.: 21, SEQ ID NO.: 22, SEQ ID NO.: 23, SEQ ID NO.: 64, SEQ ID NO.: 65, SEQ ID NO.: 66, SEQ ID NO.: 67, SEQ ID NO.: 69, SEQ ID NO.: 70, SEQ ID NO.: 71 and combinations thereof.

**25.** A method of producing aflibercept MiniTrap from a clarified harvest of a cell cultured in a chemically defined medium (CDM), comprising:

- (a) binding aflibercept from said clarified harvest to a first capture chromatography, wherein said first capture chromatography is Protein A resin;
- (b) eluting said aflibercept of step (a) and subjecting said aflibercept to enzymatic cleavage to remove its Fc domain thereby forming MiniTrap;
- (c) subjecting (b) to a second capture chromatography, wherein said second capture chromatography step is subjected to one or more washes, and wherein a first flowthrough fraction comprises MiniTrap, wherein said flowthrough fraction has  $b^*$  value of more than 0.5 when protein concentration is normalized to 5.0 g/L;
- (d) subjecting said first flowthrough fraction of step (c) to anion exchange chromatography (AEX); and
- (e) washing said AEX column of step (d), wherein said MiniTrap is collected in a second flowthrough fraction

and has a  $b^*$  value, and wherein said  $b^*$  value is lower than the  $b^*$  value in (c) when protein concentration is normalized to 5.0 g/L.

**26.** The method of claim **25**, wherein the pH of both said equilibration and wash buffers for the AEX column can be from about 7.0 to about 8.6.

**27.** The method of claim **25**, wherein said enzymatic cleavage to remove the Fc domain from aflibercept to generate MiniTrap uses an immunoglobulin-degrading enzyme of *Streptococcus pyogenes* (IdeS).

**28.** The method claim of **27**, wherein said IdeS include a polypeptide having an amino acid sequence as set forth in the group consisting of SEQ ID NO.: 2, SEQ ID NO.: 3, SEQ ID NO.: 4, SEQ ID NO.: 5, SEQ ID NO.: 6, SEQ ID NO.: 7, SEQ ID NO.: 8, SEQ ID NO.: 9, SEQ ID NO.: 10, SEQ ID NO.: 11, SEQ ID NO.: 12, SEQ ID NO.: 13, SEQ ID NO.: 14, SEQ ID NO.: 15, SEQ ID NO.: 16 and combinations thereof.

**29.** The method of claim **25**, wherein said clarified harvest comprises one or more aflibercept variants, wherein said variants have at least one oxidized amino acid residue selected from group consisting of methionine, tryptophan, histidine, phenylalanine, tyrosine and a combination thereof.

**30.** The method of claim **25**, further comprising after binding aflibercept from said clarified harvest, subjecting aflibercept to one or more further chromatographic steps selected from the group consisting of: cation exchange chromatography, hydrophobic interactive chromatography, size exclusion chromatography and a combination thereof.

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